Multiple anthelmintic resistance in Haemonchus contortus isolated from South African Boer goats in Switzerland

Schnyder, Manuela ; Torgerson, Paul R ; Schönmann, M ; Kohler, L ; Hertzberg, H

DOI: https://doi.org/10.1016/j.vetpar.2004.12.010

Multiple anthelmintic resistance in a field strain of *Haemonchus contortus* in South African Boer goats in Switzerland.

M. Schnyder[a], P.R. Torgerson[a], M. Schönmann[b], L. Kohler[a], H. Hertzberg[a,*]

[a] Institute of Parasitology, University of Zurich, Winterthurerstrasse 266a, CH-8057 Zurich, Switzerland

[b] Extension and health service for small ruminants (ESSR), CH-3360 Herzogenbuchsee

* Corresponding author. Tel.: +41-1-63-58516; fax: +41-1-63-58907. *E-mail address:* hubertus.hertzberg@access.unizh.ch

Abstract

Anthelmintic resistance was suspected in a flock in the canton of Zurich, Switzerland, into which South African Boer goats were previously imported. A strain of *Haemonchus contortus* was isolated from this herd for further investigations. Twenty sheep were allocated into one control group and three treatment groups to determine the efficacy of mebendazole, ivermectin and moxidectin using the faecal egg count reduction test (FECRT) and a controlled slaughter trial. The faecal egg count reduction percentage (FECR%) and worm burden reduction were compared using four different mathematical methods. For both tests this included the use of a maximum likelihood model where the variance of the egg counts or worm burdens were modelled using the negative binomial distribution. There was agreement in declaring high resistance levels of the anthelmintics mebendazole and ivermectin among all methods. This is the first description of resistance of gastrointestinal nematodes to one of the macrocyclic lactones in small ruminants in Switzerland. Resistance to moxidectin was suggested by 3 of the 4 mathematical techniques used for the worm burden reduction. However, with the FECRT only the negative binomial model suggested the presence of resistance against moxidectin. The use of the negative binomial distribution in statistical models appears to be more sensitive in detecting anthelmintic resistance by the FECRT, results that were confirmed by postmortem examination. The results are discussed in relation to the importance of detecting low levels of anthelmintic resistance in gastrointestinal nematodes of small ruminants in order to preserve the efficacy of important anthelmintic therapeutics.

Index keywords: Small ruminants; Macrocyclic lactone and benzimidazole resistance, Maximum likelihood model.

1. Introduction

The occurrence of anthelmintic resistance (AR) in nematodes of small ruminants has become a problem of major concern throughout the world (Conder and Campbell, 1995). It has been demonstrated that under-dosing of anthelmintics may be an important contributory factor for the development of AR (Conder and Campbell, 1995); (Escudero et al., 1999). In addition, goats show a different metabolic and pharmacokinetic profile compared to sheep (Conder and Campbell, 1995) (Escudero et al., 1999) with lower bioavailability in goats especially after oral administration (Escudero et al., 1999). Consequently macrocyclic lactone resistance appears to emerge rapidly in this species and can then spread readily to sheep (Escudero et al., 1999). Therefore particular attention to AR in goats must be given.

Resistance to one or more of the broad spectrum anthelmintics including macrocyclic lactones has been reported for goat nematodes on numerous occasions from every continent (Waller, 1997a); (Gopal et al., 1999); (van Wyk et al., 1999); (Hertzberg and Bauer, 2000); (Zajac Anne and Gipson Terry, 2000); (Terrill Thomas et al., 2001); (Veale, 2002); (Chandrawathani et al., 2003). The reports of AR in Europe mainly concern resistance against benzimidazoles (BZ), with local reports of levamisol resistance and just a few cases of ivermectin-resistance (Hertzberg and Bauer, 2000) from Denmark and the United Kingdom. Imported resistance has been reported twice in Europe. (Himonas and Papadopoulos, 1994) described a case of BZ resistance in sheep nematodes imported from the United Kingdom and France into Greece and (Varady et al., 1993) reported a case of multiple resistant trichostrongyle population in Slovakia imported from New Zealand with angora goats. This study describes the first case of an ivermectin-resistant *Haemonchus contortus*-strain in Switzerland, isolated from a flock into which South African Boer goats had been previously imported, and assesses multiple AR against mebendazole, ivermectin and moxidectin of the same strain.

The faecal egg count reduction test (FECRT) is still the most commonly used technique for the detection of AR, but different methods to calculate and interpret the results of the faecal egg count reduction percentage (FECR%) have been used and these can influence the decision taken on the continued use of an anthelmintic on a farm (Maingi et al., 1996). The FECRT still needs to be refined or alternative and more specific methods of detecting AR established. The use of parametric statistical techniques to analyse data from parasite infections is inappropriate, because such data rarely, if ever, follow a normal distribution (Barger, 1985), even in experimental infections. In order to overcome this problem, log transformation of the data before analysis has been recommended. However, for a direct comparison between different laboratories the same method of transforming the data has to be applied (Dash et al., 1988). The currently used method is to apply the transformation y = log (egg count + 1). Nevertheless, even this transformation often fails to normalise the distribution, especially when it is highly skewed (Wilson and Grenfell, 1997). In addition, type 1 errors are likely to be common and type 2 errors increased when using a log-transformed method (Wilson et al., 1996).

The World Association for the Advancement of Veterinary Parasitology (WAAVP) advises the controlled slaughter test as the most reliable method for evaluating anthelmintic activity in ruminants (Wood et al., 1995). This paper investigates the use of a maximum likelihood model, based on the negative binomial distribution, to calculate FECR% and worm burden reduction in a controlled slaughter trial and compares this with other recommended methods. Parasite burdens in hosts and their associated faecal egg counts are nearly always highly aggregated, and the negative binomial distribution is the most frequently used distribution to model this aggregation. However, with such an aggregated distribution, there is always a higher probability that the sample mean will underestimate the true population mean then over estimate it (Pacala and Dobson, 1988) and this probability increases the greater the aggregation within the host population. By

explicitly modelling the worm burdens and faecal egg counts as a negative binomial distribution, the results of this study suggest that maximum likelihood techniques are more sensitive in detection of possible resistance. Furthermore, they can indicate the presence of resistant parasites using FECRT that would otherwise have only been confirmed in a slaughter trial.

2. Materials and methods

2.1. Parasite strain

An isolate was obtained from a flock into which South African Boer goats were previously imported. The goats, living on a farm in the canton of Zurich, Switzerland, had clinical evidence of anthelmintic resistance. Faecal samples were obtained from these animals, cultured and infective larvae obtained. A previously helminth-free sheep was infected with these larvae and faeces collected when the infection became patent. These faeces were cultured for the differentiation of third stage larvae (L3) and to obtain infective larvae for subsequent experimental infection.

2.2. Experimental design

Twenty 5 to 6-month-old white alpine mountain sheep of mixed sex with a mean body weight (BW) of 32.6 kg were reared under conditions that minimized helminth infections. Before experimental infection, animals were treated with pyrantel tartrate (25 mg kg^{-1} BW) to eliminate any possible infections with gastrointestinal nematodes. Each sheep was subsequently infected orally with 15000 freshly harvested infective *H. contortus* L3 of the isolate obtained from the goats described above. At twenty-two days post infection, faecal egg counts confirmed the presence of a patent infection (Table 1) using a modified McMaster technique according to Schmidt (1971). The lower detection limit in this method is 50 EPG. Animals were randomly allocated into one control group (CON) and three

treatment groups, with 5 animals in each group, held in separate pens. At this time, mebendazole (MEB, Ovitelmin®, Janssen Pharmaceuticals, 20 mg kg^{-1} BW administered orally), ivermectin (IVM, Ivomec®, Merial, 0.2 mg kg^{-1} BW administered subcutaneously) and moxidectin (MOX, Cydectin®, Wyeth-AHP Switzerland, 0.2 mg kg^{-1} BW administered orally) in dosages according to the manufacturer's recommendation and based on individual BW were administered to each of the three treatment groups (day 0). FECs were repeated on all sheep on day 4 and on the day of slaughtering (day 6). The worm burden of each sheep was determined after slaughter by separate collection of small intestines and abomasa. Aliquots of 1/10 were taken for adult worm counts and identification.

2.3. Parasitological investigations

An egg hatch assay (EHA) was performed with nematode eggs isolated from pooled faecal samples from the goats. Eggs with an LD50 value in excess of 0.1 μg thiabendazole (TBZ) ml^{-1} are indicative of BZ resistance in accordance with the method described in the WAAVP guidelines for detection of AR (Coles et al., 1992).

2.4. Data analysis

Data from the EHA were analysed to determine the LD50, i.e. concentrations of TBZ required to inhibit 50% of eggs from hatching. The percentage of eggs failing to hatch at each concentration of TBZ, after correction for natural mortality, were transformed to logits and used to generate dose response lines for the isolate using a logit program. From these, the LD50 values were calculated. Resistance is declared if the LD50 is above 0.1 μg TBZ ml^{-1} (Coles et al., 1992).

Data from the FECRT were entered on a spreadsheet (Microsoft Excel, Microsoft Corp. WA) and the FECR% and the 95% confidence intervals for the reduction were calculated

according by various methods. The WAAVP recommendations for detection of anthelmintic resistance (Coles et al., 1992) recommend that the FECR% is calculated as:

$$FECR\% = 100 \times \left(1 - \frac{Xt}{Xc}\right) \quad (1)$$

where *Xt* and *Xc* are the arithmetic mean EPG in the treated (*t*) and non-treated control (*c*) groups. Resistance is considered to be present if the FECR is less than 95% and the lower 95% confidence limit for the reduction is less than 90%. If only one of these conditions are met, resistance is suspected.

(Presidente, 1985) and (Dash et al., 1988) recommend that the FECR% reduction is calculated as:

$$FECR\% = 100 \times \left(1 - \frac{Xt2}{Xt1} \times \frac{Xc1}{Xc2}\right) \quad (2)$$

where (*Xc*) and (*Xt*) are the geometric mean (Presidente, 1985) or arithmetic mean (Dash et al., 1988) EPG for control (c) and treated (t) groups before ($_1$) and after ($_2$) treatments respectively. According to (Dash et al., 1988) resistance is declared if the FECR% is less than 80%, while (Presidente, 1985) stated that treatment with modern broad-spectrum anthelmintics should result in a FECR% of > 95%.

In the controlled slaughter test, according to the WAAVP recommendations (Wood et al., 1995), the percent efficacy (%E) of an anthelmintic treatment against a given parasite species in each treatment group is:

$$\%E = \left(\frac{Xcg - Xtg}{Xcg}\right) \times 100\,, \quad (3)$$

where *Xtg* is the geometric mean of the treatment group and *Xcg* the geometric mean of the control group. A compound should be declared effective only when effectiveness stands at 90% or above (using pooled data, when appropriate) and there is a statistically significant difference in parasite numbers between control and treated animals.

The percent efficacy (%E) was additionally calculated with the same formula (1) utilised by (Coles et al., 1992) for the FECR%, as suggested by (Hong et al., 1996) and (Watson et al., 1996), and with a method utilised by (Borgsteede et al., 1996):

$$\%E = 100 - \left(\frac{Xt \times 100}{Xc} \right), \quad (4)$$

using arithmetic means. A value below which resistance has to be considered is not given. For both, calculation of FECR% and %E, a maximum likelihood model was also adopted.

Parasite abundance in infected animals usually follows an aggregated distribution in naturally infected animals (Wilson, 1996). In many instances, due, for examples, to variations in innate resistance, even artificially infected animals have a non-uniform distribution. Therefore the mean and variance of the parasite burden found at necropsy and the parasite faecal eggs counts from each group were compared. Evidence of aggregation, defined as the variance being greater than the mean, was confirmed in all groups. The frequency distributions of the parasite burdens and faecal egg counts were consequently modelled as a negative binomial distribution with the two parameters of arithmetic mean and negative binomial constant *k*. Thus the probability of the number of parasites *s* for each observation o_i given the arithmetic mean *M* and negative binomial constant *k* is given as:

$$\Pr\{o_i = s\} = \frac{\Gamma(k+s)}{\Gamma(k)s!}\left(\frac{M}{k+M}\right)^s\left(\frac{k}{k+M}\right)^k \quad (5)$$

where Γ is the gamma function.

All the observations were fitted using this likelihood function and the total likelihood was:

$$\prod_{i=1}^{n} \Pr\{o_i = s\}$$

A likelihood profile (Venzon and Moolgavkar, 1988), and hence probability density distributions of the arithmetic means given the data, were found with the negative binomial likelihood function by means of the likelihood profile function of the Excel™ (Microsoft Corp, Redmond WA) PopTools add on (CSIRO Australia). Confidence limits for reductions in the mean worm burden or faecal egg counts were calculated using Monte-Carlo methods. Using Crystal Ball software (Decisoneering Corp, Denver CO) random samples were drawn from the probability distribution of the arithmetic mean, generated by the likelihood profile, of the control and test groups. For each pair of random samples the efficacy was calculated using the formula of (Coles et al., 1992). This process was repeated 10000 times. The lower 2.5 percentile and upper 97.5 percentile of the results of these 10000 iterations are accurate estimates of the 95% confidence limits of the anthelmintic efficacy assuming a negative binomial model of parasite burden and faecal egg counts.

3. Results

The LD50 values obtained in the EHA from the strain isolated from the goat farm in the egg hatch test was 0.72 μg TBZ ml^{-1}, demonstrating the presence of BZ resistant nematodes. Faecal cultures from pooled faecal samples of the South African Boer goats,

after culture and L3-infection of a single sheep, had strongylid larvae composed of a mean larval proportion of 86% *H. contortus*, 8% *Trichostrongylus* sp. and 6% *Ostertagia* sp.

The post-treatment faecal cultures obtained from the slaughtered sheep showed a strongylid larvae composition with almost exclusively *H. contortus*: 100% in the CON and MOX groups, 100% also in the MEB group with an additionally presence of *Strongyloides sp.*, and 99% *H. contortus* and 1% *Ostertagia spp*. in the IVM group.

The results of the FEC and the controlled slaughter test of the control group are shown in Table 1. Worm burden comprehend only adult worms found in the abomasa. Small amounts of adult worms were also found in small intestines from sheep of the CON and the MEB groups. In one sheep from the CON group *T. colubriformis* was found, but all other isolated adult male worms were identified as *H. contortus*.

There was agreement in declaring resistance of *H. contortus* among all four methods of calculating FECR% and all four methods of calculating %E of the anthelmintics MEB (Table 2) and IVM (Table 3).

The FECR% obtained with MOX (Table 4) on the day of slaughtering showed different results. Susceptibility was declared according to the WAAVP (Coles et al., 1992) method (the FECR% is more than 95% and the lower confidence limit for the reduction is more than 90%) and the method proposed by (Dash et al., 1988) (FECR% more than 80%). The FECR% calculated according to the method of (Presidente, 1985) is located on the cut-off level for declaring resistance. Only the FECR% calculated with the use of the maximum likelihood model showed a suspected resistance as the lower confidence limit was less than 90%. The results of the controlled slaughter test show high efficacy of MOX compared to MEB and IVM, but the %E calculated with three methods (Table 4) and the lower confidence limit calculated with the WAAVP method (Coles et al. 1992) and the maximum likelihood models (58.1 and 58.5 respectively) indicated resistance to MOX.

4. Discussion

The results of the FECRT carried out and interpreted by four different methods, the LD50 values obtained in the EHA and the results of the worm burden reduction calculated by four different methods provided evidence of resistance to BZ of the strain isolated from the South African Boer goats. BZ resistance has already been described in Switzerland (Hertzberg et al., 2000), with a prevalence of 91% in goats (Meyer, 2001). Resistance to IVM has been highly suspected by the data from the FECRT (ranging from 39.8% to 55.7% between the four different methods) and confirmed by a worm burden reduction result of 61%, with agreement between all four methods. The lower confidence limit calculated according to the WAAVP recommendations (Coles et al., 1992) is higher compared with the one calculated using the negative binomial model (43.4% and 38.8% respectively), but there is agreement in declaring resistance. In the case of MOX, there were disagreements in declaring resistance with the FECRT between the different methods. With the methods by (Coles et al., 1992) and (Dash et al., 1988) the *H. contortus*-strain isolated from the South African Boer goats is declared susceptible against MOX, with the (Presidente, 1985) method, obtaining a FECR% of 94.9%, the strain is suggested to be considered on a level of suspected resistance (McKenna, 1990). In fact, a lower confidence limit of 81.3% calculated with the maximum likelihood model confirms a suspect case of resistance. The use of a maximum likelihood model here takes into account the probability distribution of parasite data and calculates the confidence limits of the mean accordingly. (Wilson, 1996) demonstrated that the use of models where the error structure of the data is explicitly defined as a negative binomial distribution is much less likely to produce type 1 or type 2 statistical errors than other analytical techniques. This method demonstrates that suspected resistance can be detected with FECs which may have been missed using the method recommended by the WAAVP. Furthermore, the possibility of resistance against MOX was confirmed by post-mortem worm counts,

demonstrating the value of this analytical method. The sample mean has a higher probability of underestimating the true mean when parasite burdens are aggregated (Pacala and Dobson, 1988). Hence the true faecal count reduction has a high probability of being less the observed reduction, particularly when small sample sizes are used. The negative binomial model accounts for this and hence gives asymmetric confidence intervals. The wide confidence interval models the uncertainty inherent in the small sample sizes. These confidence intervals would narrow if the minimum groups size was increased to greater numbers than those recommended by the Guidelines for international harmonisation of anthelmintic efficacy (Vercruiysse et al., 2001).

The controlled slaughter test, according to the WAAVP recommendations (Wood et al., 1995), is the most reliable method for evaluating anthelmintic activity in ruminants, but due to its costs and complexity not applicable for routine diagnosis. In contrast to the controlled slaughter trial, FECRT uses few resources, is easily performed and is also applicable in the evaluation of the performance of any anthelmintic (Maingi et al., 1996). Fortunately there is good correlation in the relationship between the reduction in worm burden and FECRT following anthelmintic treatment (McKenna, 1990). However, reductions in faecal egg counts tend to be greater than the reductions in worm burden (McKenna, 1990). Using the maximum likelihood model, there was a greater sensitivity in the detection of resistance in this study using reductions in egg counts than the statistical methods recommended by the WAAVP (Coles et al., 1992)(Coles et al., 1992). The two criteria to declare resistance against MOX according to WAAVP (Coles et al., 1992)(Coles et al., 1992) are met: FECR% is less than 95% and the lower 95% confidence level is less than 90%. The confirmation of suspected MOX-resistance is given by the results of the worm burden reduction. The method by Coles et al. (1992)(Coles et al., 1992) and the method using a maximum likelihood model gave very similar results in %E (90.7% both) and in confidence limits (58.1-98.0% and 58.5-96.7% respectively). Discrepancies in declaring

resistance can influence the decision taken on the continued use of an anthelmintic on a farm. (Maingi et al., 1996) compared four different methods to calculate FECR% and stated that use of any of the four methods is likely to influence the decision taken concerning the resistance status on a farm when the level of AR is low. In this study, all test and methods used to check the resistance status agreed in declaring a high level of BZ and IVM resistance, while there were discrepancies in declaring resistance for MOX. The only method that suggested MOX resistance by the FECRT, involving a control group and therefore consistent with the International Anthelmintic Efficacy Guidelines (Vercruysse et al., 2001), was by modelling the FECs using a negative binomial model. Some statistical packages can now be used in which the error variance can be expressed explicitly. However, in this paper the analysis was undertaken in Excel with the relevant formulae (eg equation 5) written into individual cells to give the likelihood of the data, given the model and the total likelihood maximised using the solver add on. With the increasing sophistication of computer hardware and software, such methods should be more widely used.

The presence of multiple AR including resistance against IVM has been described recently from different continents: from Malaysia (Chandrawathani et al., 2003) in sheep and goats, from the USA (Zajac Anne and Gipson Terry, 2000) and from Kenya in goats (Mwamachi et al., 1995).

Anthelmintic dosages for goats have been the source of much discussion because of the different metabolism of goats compared to sheep ((Escudero et al., 1999)). However, there is agreement that goats require higher doses of anthelmintics (Conder and Campbell, 1995). Higher dosages for anthelmintics registered for goats should be mentioned in the package instructions.

There is even greater controversy about the use of MOX for goats. MOX provided a high degree of protection against reinfection with *H. contortus* and *T. circumcincta*, but no

useful effect against *T. colubriformis* (Torres-Acosta and Jacobs, 1999). Beyond it, is MOX not approved for use in goats (Baynes et al., 2000) and in New Zealand there is a contraindication statement on the MOX label for use in goats (Cobb and Murphy, 1995). Despite there being statement on the label that MOX is contraindicated in goats, farmers in New Zealand are reported to use MOX "off-label" in the face of IVM resistance (Watson et al., 1996). Leathwick (1995) reported an instance where MOX failed to control an IVM resistant strain of *Ostertagia* species in goats. Before use of MOX, where IVM resistance is suspected, efficacy needs to be established. Finally, Escudero et al. (1999) recommended pharmacokinetic data for goats to be produced to avoid extrapolating dosages from other species. Low doses can lead to ineffective therapeutic levels and therefore increase chances of development of resistant parasites. In this study, the resistant *H. contortus* strain obtained from South African Boer goats was transmitted to sheep . The results support the observations of Watson et al. (1996): an *Ostertagia* spp. strain with IVM-MOX side resistance isolated from Boer goats was transmitted to sheep. The conclusion was that where IVM resistance is present, the development of MOX resistance is expected to ensue.

Caution should be exercised when importing livestock from countries with widespread development of multiple AR (Waller, 1997b). Varady et al. (1993) and Himonas and Papadopoulos (1994) already reported from AR imported with transfer of life stock in Europe. Resistance to any component of the macrocyclic lactones has never been found before in Switzerland. This is the first description of a *H. contortus* strain resistant against exponents of the group of the macrocyclic lactones (IVM and MOX) in Switzerland. There is no history of excessive use of IVM in the investigated flock, nor of any use of MOX. Imported South African Boer goats are therefore the likely origin of the resistant parasites reported in this study. Varady et al. (1993) recommended the introduction of legislation to

control this problem. Likewise, Himonas and Papadopoulos (1994) suggested that animals originating from countries with AR should at least be investigated before export/import.

Acknowledgements

We gratefully acknowledge the owners of the Boer goats furnishing the resistant H. contortus strain for their collaboration. We would also like to thank Prof. P. Deplazes of the University of Zurich for fruitful discussions and Dr. med. vet. M. Ebeid for his help in sample collection.

References

Barger, I.A., 1985. The statistical distribution of trichostrongylid nematodes in grazing lambs. Int. J. Parasitol. 15, 645-649.

Baynes, R.E., Payne, M., Martin-Jimenez, T., Abdullah, A.R., Anderson, K.L., Webb, A.I., Craigmill, A., Riviere, J.E., 2000. Extralabel use of ivermectin and moxidectin in food animals. J. Am. Vet. Med. Assoc. 217, 668-671.

Borgsteede, F.H.M., Pekelder, J.J., Dercksen, D.P., 1996. Anthelmintic resistant nematodes in goats in the Netherlands. Vet. Parasitol. 65, 83-87.

Chandrawathani, P., Waller, P.J., Adnan, M., Hoglund, J., 2003. Evolution of high-level, multiple anthelmintic resistance on a sheep farm in Malaysia. Trop. An. Health Prod. 35, 17-25.

Cobb, R.M., Murphy, A.W., 1995. Use of moxidectin in goats in New Zealand. Vet. Rec. 137, 496-496.

Coles, G.C., Bauer, C., Borgsteede, F.H., Geerts, S., Klei, T.R., Taylor, M.A., Waller, P.J., 1992. World Association for the Advancement of Veterinary Parasitology

(W.A.A.V.P.): methods for the detection of anthelmintic resistance in nematodes of veterinary importance. Vet. Parasitol. 44, 35-44.

Conder, G.A., Campbell, W.C., 1995. Chemotherapy of nematode infections of veterinary importance, with special reference to drug resistance. In: Baker, J.R., Muller, R., Rollinson, D. (Eds.) Advances in Parasitology, Vol 35. Academic Press Ltd, pp. 1-84.

Dash, K.M., Hall, E., Barger, I.A., 1988. The role of arithmetic and geometric mean worm egg counts in faecal egg count reduction tests and in monitoring strategic drenching programs in sheep. Aust. Vet. J. 65, 66-68.

Escudero, E., Carceles, C.M., Diaz, M.S., Sutra, J.F., Galtier, P., Alvinerie, M., 1999. Pharmacokinetics of moxidectin and doramectin in goats. Res. Vet. Sci. 67, 177-181.

Gopal, R.M., Pomroy, W.E., West, D.M., 1999. Resistance of field isolates of *Trichostrongylus colubriformis* and *Ostertagia circumcincta* to ivermectin. Int. J. Parasitol. 29, 781-786.

Hertzberg, H., Bauer, C., 2000. Anthelmintic resistance in gastrointestinal *Strongylidae* in sheep and goats: new data on prevalence, epidemiology, preventive measures and alternatives to anthelmintic drugs. Berl. Muench. Tieraerztl. Wschr. 113, 122-128 (in German).

Hertzberg, H., Rossmann, J., Kohler, L., Willi, U., 2000. Benzimidazole-resistance in gastrointestinal nematodes of sheep and goats in Switzerland. Wien. Tierarztl. Monatsschr. 87, 3-9 (in German).

Himonas, C., Papadopoulos, E., 1994. Anthelmintic resistance in imported sheep. Vet. Rec. 134, 456.

Hong, C., Hunt, K.R., Coles, G.C., 1996. Occurrence of anthelmintic resistant nematodes on sheep farms in England and goat farms in England and Wales. Vet. Rec. 139, 83-86.

Leathwick, D.M., 1995. A case of moxidectin failing to control ivermectin resistant *Ostertagia* species in goats. Vet. Rec. 136, 443-444.

Maingi, N., Bjorn, H., Thamsborg, S.M., Bogh, H.O., Nansen, P., 1996. A survey of anthelmintic resistance in nematode parasites of goats in Denmark. Vet. Parasitol. 66, 53-66.

McKenna, P.B., 1990. The detection of anthelmintic resistance by the faecal egg count reduction test: An examination of some of the factors affecting perfomance and interpretation. N. Z. Vet. J. 38, 142-147.

Meyer, A., 2001. Verbreitung von Benzimidazol-Resistenzen bei den Trichostrongyliden von Schafen und Ziegen in der Schweiz. Inaugural-Dissertation zur Erlangung der Doktorwürde der Veterinärmedizinischen Fakultät der Universität Zürich. Zürich.

Mwamachi, D.M., Audho, J.O., Thorpe, W., Baker, R.L., 1995. Evidence for multiple anthelmintic resistance in sheep and goats reared under the same management in coastal Kenya. Vet. Parasitol. 60, 303-313.

Pacala, S. W., Dobson, A. P., 1988. The relationship between the numbers of parasites/host and host age: population dynamic causes and maximum likelihood estimation. Parasitology. 96, 197-210.

Presidente, P.J.A., 1985. Methods for detection of resistance to anthelmintics, In: Waller, N.A.a.P.J. (Ed.), Resistance in nematodes to anthelmintic drugs. C.S.I.R.O. Division of Animal Health, Glebe, N.S.W., Australia, pp. 13-27.

Schmidt, U., 1971. Parasitologische Kotuntersuchung durch ein neues Verdünnungsverfahren. Tieraerztl. Umsch. 26, 229-230 (in German).

Terrill, Th. H., Kaplan, R, M., Larsen, M., Samples, O. M., Miller, J. E., Gelaye, S., 2001. Anthelmintic resistance on goat farms in Georgia: Efficacy of anthelmintics against gastrointestinal nematodes in two selected goat herds. Vet. Parasitol. 97, 261-268.

Torres-Acosta, J.F., Jacobs, D.E., 1999. Duration of activity of oral moxidectin against *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis* in goats. Vet. Rec. 144, 648-649.

van Wyk, J.A., Stenson, M.O., van der Merwe, J.S., Vorster, R.J., Viljoen, P.G., 1999. Anthelmintic resistance in South Africa: Surveys indicate an extremely serious situation in sheep and goat farming. Onderstepoort J. Vet. Res. 66, 273-284.

Varady, M., Praslicka, J., Corba, J., Vesely, L., 1993. Multiple anthelmintic resistance of nematodes in imported goats. Vet. Rec. 132, 387-388.

Veale, P.I., 2002. Resistance to macrocyclic lactones in nematodes of goats. Aust. Vet. J. 80, 303-304.

Venzon, D. J., Moolgavkar, S. H., 1988. A method for computing profile-likelihood based confidence intervals. Appl. Statistics. 37, 87-94.

Vercruysse, J., Holdsworth, P., Letonja, T., Barth, D., Conder, G., Hamamoto, K., Okano, K., 2001. International harmonisation of anthelmintic efficacy guidelines. Vet. Parasitol. 96, 171-193.

Waller, P.J., 1997a. Anthelmintic resistance. Vet. Parasitol. 72, 391-405.

Waller, P.J., 1997b. Nematode parasite control of livestock in the tropics/subtropics: the need for novel approaches. Int. J. Parasitol. 27, 1193-1201.

Watson, T.G., Hosking, B.C., Leathwick, D.M., McKee, P.F., 1996. Ivermectin-moxidectin side resistance by Ostertagia species isolated from goats and passaged to sheep. Vet. Rec. 138, 472-473.

Wilson, K., Grenfell, B. T., 1997.Generalized linear modelling for parasitologists. Parasitol. Today 13, 33-38.

Wilson, K., Grenfell, B.T., Shaw, D.J., 1996. Analysis of aggregated parasite distributions: a comparison of methods. Funct. Ecol. 10, 592-601.

Wood, I.B., Amaral, N.K., Bairden, K., Duncan, J.L., Kassai, T., Malone, J.B., Jr., Pankavich, J.A., Reinecke, R.K., Slocombe, O., Taylor, S.M., et al., 1995. World Association for the Advancement of Veterinary Parasitology (W.A.A.V.P.): second edition of guidelines for evaluating the efficacy of anthelmintics in ruminants (bovine, ovine, caprine). Vet. Parasitol. 58, 181-213.

Zajac, A. M., Gipson, T. A., 2000. Multiple anthelmintic resistance in a goat herd. Vet. Parasitol. 87, 163-172.

Table 1

Faecal egg counts (FEC) and post mortem worm burdens recorded in the control group infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs were recorded 22 days post infection (day 0) and 6 days later (day 6) on the day of slaughtering.

Control group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	1400	4600	3632
Variance	437500	3921250	529170
95% negative binomial CL	931-2245	3030-7470	2995-4461
Geometric mean	1282	4194	3570
95% CL of geometric mean	852-1930	2691-6535	2964-4299

CL = confidence limits.

Table 2

Faecal egg counts (FEC) and post mortem worm burdens recorded in the mebendazole treated group infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs were recorded 22 days post infection (day 0) and 6 days later (day 6) on the day of slaughtering. Percent faecal egg count reduction (FECR%) and percent worm burden reduction efficacy (%E) were calculated according to four methods on the 5 sheep.

Mebendazole group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	1022	1800	1642
Variance	2256670	2393750	224020
95% negative binomial CL	412-3749	973-3901	1286-2142
Geometric mean	535	1428	1596
95% CL of the geometric mean	196-1456	762-2674	1273-2000
FECR% and %E (Coles et al., 1992)		60.9	54.8
95% CL (Coles et al., 1992)		-13.6-86.5	33.3-69.4
FECR% (Dash et al., 1988)		46.4	
FECR% (Presidente, 1985)		18.4	
%E (Wood et al., 1995)			55.3
%E (Borgsteede et al., 1996)			54.8
FECR% and %E (maximum likelihood)		60.8	54.7
95% CL (maximum likelihood)		8.9-81.8	29.8-70.2

CL = confidence limits.

Table 3

Faecal egg counts (FEC) and post mortem worm burdens recorded in the ivermectin treated group infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs were recorded 22 days post infection (day 0) and 6 days later (day 6) on the day of slaughtering. Percent faecal egg count reduction (FECR%) and percent worm burden reduction efficacy (%E) were calculated according to four methods on the 5 sheep.

Ivermectin group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	1700	2770	1430
Variance	1081250	4959500	136550
95% negative binomial CL	1083-2886	1506-5947	1133-1840
Geometric mean	1523	2209	1394
95% CL of geometric mean	999-2322	1166-4186	1118-1737
FECR% and %E (Coles et al., 1992)		39.8	60.6
95% CL (Coles et al., 1992)		65.6-78.1	43.4-72.6
FECR% (Dash et al., 1988)		50.4	
FECR% (Presidente, 1985)		55.7	
%E (Wood et al., 1995)			61.0
%E (Borgsteede et al., 1996)			60.6
FECR% and %E (maximum likelihood)		39.8	60.5
95% CL (maximum likelihood)		-36.1-71.1	38.8-73.9

CL: confidence limits.

Table 4

Faecal egg counts (FEC) and post mortem worm burdens recorded in the moxidectin treated group infected with a suspected anthelmintic resistant strain of *H. contortus*. FECs were recorded 22 days post infection (day 0) and 6 days later (day 6) on the day of slaughtering. Percent faecal egg count reduction (FECR%) and percent worm burden reduction efficacy (%E) were calculated according to four methods on the 5 sheep.

Moxidectin group (n=5)	FEC		worm burden
	day 0	day 6	day 6
Arithmetic mean	360	110	336
Variance	154250	11750	204730
95% negative binomial CL	171-960	31-898	123-1486
Geometric mean	250	42	147
95% CL of geometric mean	114-548	6-294	38-573
FECR% and %E (Coles et al., 1992)		97.6	90.7
95% CL[c] (Coles et al., 1992)		92.1-99.3	58.1-98.0
FECR% (Dash et al., 1988)		90.7	
FECR% (Presidente, 1985)		94.9	
%E (Wood et al., 1995)			95.9
%E (Borgsteede et al., 1996)			90.7
FECR% and %E (maximum likelihood)		97.6	90.7
95% CL[c] (maximum likelihood)		81.3-99.3	58.5-96.7

CL= confidence limits.